PATENT COOPERATION TREATY

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

Davies Collison Cave
Level 15
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MELBOURNE VIC 3000

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

JGC

Date of mailing *day/month/year* 20 JUL 2004

Applicant's or agent's file reference
12182600/JGC

IMPORTANT NOTIFICATION

International Application No.	International Filing Date	Priority Date
PCT/AU2003/000351	20 March 2003	20 March 2002

Applicant
THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translations to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide

Name and mailing address of the IPEA/AU	Authorised officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	G. D. HEARDER Telephone No. (02) 6283 2553

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
12182600/JGC		
International Application No.	International Filing Date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/AU2003/000351	20 March 2003	20 March 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] C07D 307/86, 409/12, 417/12, 405/12, A61K 31/343, 31/381, 31/427, 31/433, 31/443, 31/513, 31/497, 31/501, A61P 37/00, 29/00

Applicant

THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [X] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of the report
19 September 2003	16 July 2004
Name and mailing address of the IPEA/AU	Authorised Officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	G. D. HEARDER Telephone No. (02) 6283 2553

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000351

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **1, 2, 5-7, 9-48, 50, 51,** as originally filed,
pages , filed with the demand,
pages **3, 4, 8, 49,** received on **20 April 2004** with the letter of **20 April 2004**

☒ the claims, pages **52-61,** as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

☐ the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be nonobvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos: **1-10, 18-21 (as they relate to claims 1-10)**

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. **1-10, 18-21 (as they relate to claims 1-10)**

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	**11-17, 18-21 (in part)**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**11-17, 18-21 (in part)**	**YES**
	Claims		**NO**
Industrial applicability (IA)	Claims	**11-17, 18-21 (in part)**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

The following document identified in the International Search Report has been considered for the purposes of this report:

Wulff H. et al.; The Physiologist, no. 42, (1999), p A-12, abstract 5.67

Claims 11-17, 18-21 (as they relate to claims 11-17)

No individual citation or combination of citations disclose the features of these claims.

channels open, the resulting efflux of K^+ hyperpolarizes the membrane, which in turn accentuates the entry of Ca^{2+}, which is absolutely required for downstream activation events (Cahalan and Chandy 1997, *Curr. Opin. Biotechnol.* 8: 749).

The predominant voltage-gated channel in human T-lymphocytes is encoded by *Kv1.3*, a *Shaker*-related gene. *Kv1.3* has been characterised extensively at the molecular and physiological level and plays a vital role in controlling T-lymphocyte proliferation, mainly by maintaining the resting membrane potential of resting T-lymphocytes. Inhibition of this channel depolarises the cell membrane sufficiently to decrease the influx of Ca^{2+} and thereby prevents downstream activation events. Advantageously the homotetrameric *Kv1.3* channel is almost exclusively located in T-lymphocytes.

Accordingly compounds which are selective *Kv1.3* blockers are thus potential therapeutic agents as immunosuppressants for the prevention of graft rejection, and the treatment of autoimmune and inflammatory disorders. They could be used alone or in conjunction with other immunosuppressants, such as selective *IKCa1* blockers or cyclosporin, in order to achieve synergism and/or to reduce toxicity, especially of cyclosporin.

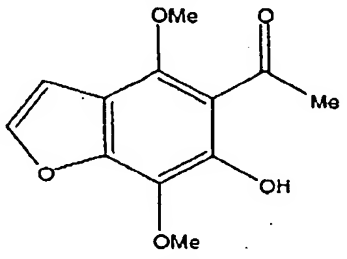
US Patent No. 5,494,895 discloses the use of a thirty-nine amino acid peptide, scorpion peptide margatoxin, as a selective inhibitor and probe of *Kv1.3* channels present in human lymphocytes, and also as an immunosuppressant. However the use of this compound is limited by its potent toxicity.

International Patent Application publication No.s WO 97/16438 and WO 09/716437, and US Patent No. 6,051,590 describe the use of the triterpene, correolide and related compounds as immunosuppressants in the treatment of conditions in mammals affected or facilitated by *Kv1.3* inhibition.

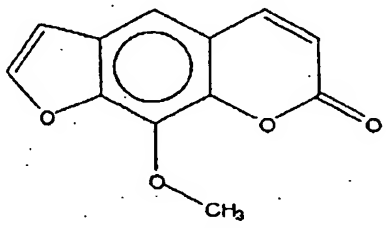
US Patent 6,077,680 describes DNA segments and proteins of derived from sea anemone species, more particularly ShK toxin from Stichodactyla helianthus. The ShK toxin was found to block *Kv1.1, Kv1.3, Kv1.4* and *Kv1.6*, but a mutant ShK-K22DAP found to selectively block *Kv1.3*.

ShK toxin has recently been shown to both prevent and treat experimental autoimmune encephalomyelitis in Lewis rats, an animal model for human multiple sclerosis (Beeton 2001,et al., *Proc. Natl. Acad. Sci. USA* 98:13942), by selectively targeting T-cells chronically activated by the myelin antigen, MBP (myelin basic protein). The same study also indicated that chronically activated encephalitogenic rat T- cells express a unique channel phenotype characterised by high expression of *Kv1.3* channels (approximately 1500 per cell) and low numbers of *IKCa1* channels (approximately 120 per cell). This channel phenotype is distinct from that seen in quiescent and acutely activated cells and may be a functionally relevant marker for chronically activated rat T lymphocytes.

Khellinone, a substituted benzofuran and natural product from certain plants, and 8-Methoxypsoralen (8-MOP), both commercially available products have been found to have blocking activity on the *Kv1.3* channel.



Khellinone



8-Methoxypsoralen

However, it is believed that the compounds of the invention may address one or more of these problems and thus may be more selective and/or active and/or soluble than the known divalent ligands, in addition to having good stability.

With respect to the present invention there is a Linker joined to two ligands in order to provide the compound of formula I, as earlier described. The "Linker" is a divalent spacer group that provides a space between the two aromatic rings to which it is joined of from 6 to 11 atoms when measured across the shortest route between the two aromatic rings (the bridging portion). Ideally this length should permit the active components to bridge two neighbouring binding sites.

Examples of suitable divalent spacer groups include optionally substituted alkylene groups having from 6 to 11 carbon atoms when measured across the shortest route between the two aromatic rings. One or more of the methylene ($-CH_2-$) groups in the bridging portion may be replaced with heteroatoms, such as O, S or NR^a wherein R^a is selected from hydrogen and lower alkyl. One or more of the methylene ($-CH_2-$) groups in the bridging portion may also be replaced with atoms forming part of an optionally substituted ring such as one or more optionally substituted aromatic ring or optionally substituted non aromatic ring(s). The rings may include one or more heteroatoms selected from O, S and N. The ring heteroatoms may form part of the bridging portion.

The bridging portion (including non-aromatic ring(s) forming part of the bridging portion) may include one or more unsaturated sites. An unsaturated site occurs when an ethylene moiety ($-CH_2CH_2-$) has been replaced with -CH=CH- or -C≡C-.

The spacer group may be optionally substituted by a wide range of substituents, such as those described below with reference to the definition of 'optionally substituted'. By way of a non-limiting example, the spacer group may be optionally substituted with one of more substituents selected from hydroxy, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted

In the case of known comparative example 4, it was found that this compound was so poorly soluble in aqueous media, it could not be tested for inhibition of T-cell proliferation in a cell-based assay. The comparative example 4 was thus considered to lack therapeutically use. However, the compound was surprisingly found to have good stability.

Given the poor solubility of comparative example 4, it was surprisingly found that examples 1 and 2 were soluble enough in aqueous media to be tested for inhibition of T-cell proliferation in a cell-based assay. This was unexpected given the lipophilicity of these compounds. The n-butyl fragment in example 2 is considerably more lipophilic than the phenyl group located in the equivalent position of comparative example 4 (fragment hydrophobicity value 2.5 verses 1.9 respectively).

It is thought that the improvement in aqueous solubility relates to the crystalline structure of the compounds. The Linker in example 2 is similar in length to the length of the Linker in comparative example 4, and as such it may permit similar binding of the non-linker portions to Kv1.3. However, the Linker of example 2 should have greater conformational flexibility relative to the more rigid phenyl-based linker in comparative example 4. This may have produced a compound which is inherently much less crystalline than that of the comparative example. The fact that the melting point of comparative example 4 is more than twice that of example 2 lends support to this theory.

Example 5 is also significantly more soluble in aqueous conditions than comparative example 2. It has a lower melting point, and is an oil at room temperature. The oxygen atom midway in the Linker, being polar, may further increase water solubility, though the placement of this atom appears to have caused some weakening in the binding to Kv1.3, as indicated by the decreased K_d value.